JOURNAL OF ANIMAL SCIENCE
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J Anim Sci 1981. 52:1312-1318.

MOLDY CORN IN DIETS OF SWINE

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Summary

Three trials were conducted to evaluate the effect of consumption of moldy corn on pig performance. In trial 1, four levels of zearalenone from moldy corn (0, 3, 6 and 9 ppm) were fed to 24 gilts (average weight 64 kg) for 42 days. Rate of gain, feed consumption and gain to feed ratio decreased with increasing dietary levels of moldy corn. There appeared to be some adaptation to the higher levels of moldy corn with time. The same dietary treatments fed in trial 1 were fed to 24 barrows and 24 gilts (initial weight 6.3 kg) in trial 2. In trial 3, the same levels of zearalenone fed in trials 1 and 2 were fed to 24 gilts, but a different sample of moldy corn was used. Increasing levels of moldy corn resulted in decreased rate of gain. Swelling and redness of the vulvae were observed in gilts fed diets containing 6 or 9 ppm zearalenone. Uterine weight increased with increasing levels of zearlenone in the diet, as did the thickness of the vaginal epithelium.

(**Key Words:** Moldy Corn, Zearalenone, Vomitoxin, Swine Diets.)

Introduction

Feed refusal or decreased feed intake by swine, leading to poor performance by growing pigs and reproductive problems in sows, has been observed, with moldy corn often implicated as a contributing factor.

Fusarium, a fungus which may grow on corn in the field, is reported to produce several toxins, including zearalenone, an estrogen; vomitoxin, an emetic-refusal factor, and T-2 toxin, a vesicant (Vesonder *et al.,* 1977). There is evidence to suggest that naturally contaminated corn may have a greater effect than corn that contains similar amounts of added purified toxins (Nelson *et al.,* 1973; Forsyth *et al.,* 1977). This suggests a synergistic action or the possibility of other unidentified toxic factors.

Limited information is available in the literature on the quantitative and qualitative effects of mold toxins on swine. Christensen *et al.* (1977a) stated that "when consumed by swine in amounts of more than a few parts per million, F-2 (zearalenone) causes the estrogenic syndrome." Mirocha and Christensen (1975) reported that zearalenone did not cause abortion in swine, and Christensen *et al.* (1977b) did not observe stunting when pure F-2 was added to the diet in a sufficient amount to cause severe estrogenism. Mirocha and Christensen (1975) indicated that zearalenone in concentrations between 1 and 5 ppm caused hyperestrogenism in swine, whereas Patterson *et al.* (1977) observed gilts to be clinically normal when fed 2 mg of zearalenone/kg feed throughout gestation.

There is some confusion in the literature regarding the identity of the emetic and refusal factors in corn; however, Vesonder *et al.* (1976) suggested that vomitoxin (4-deoxynivalenol) is responsible for both feed refusal and emesis. Forsyth *et al.* (1977) reported almost total refusal by pigs of corn naturally infected and containing 12 ppm of deoxynivalenol, but found that those fed corn with purified deoxynivalenol added to 16 ppm consumed considerable feed.

The experiments reported herein were conducted to provide further information about the qualitative and quantitative effects on swine of mycotoxins on corn grown in the field.

Materials and Methods

Trial 1. Twenty-four Yorkshire gilts (average weight 64 kg) were allotted to four dietary treatments (table 1) wherein dry shelled corn was replaced with moldy corn at levels formu-

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[5] This research was supported by the Ontario Ministry of Agriculture and Food and the Natural Sciences and Engineering Research Council, Canada.

TABLE 1. DIETARY TREATMENTS USED TO EVALUATE MOLDY CORN IN SWINE DIETS, TRIALS 1, 2 AND 3

Item Trial	1 and 2				3			
Level of zearalenone								
Calculated	0	3	6	9	0	3	6	9
Analysis					0	2.5	4.4	6.7
Ingredients								
Ground corn (IFN 4-02-992)	81.7	74.0	66.3	58.6	72.5	69.7	66.9	64.1
Moldy corn[a]	0	7.7	15.4	23.1	0	2.8	5.6	8.4
Soybean, seed W.O. hulls, Solv-extd. ground (IFN 5-04-612)	14.5	14.5	14.5	14.5	24.0	24.0	24.0	24.0
Limestone (IFN 6-02-632)	1.4	1.4	1.4	1.4	.9	.9	.9	.9
Calcium phosphate[b]	1.3	1.3	1.3	1.3	1.5	1.5	1.5	1.5
Salt[c]	.5	.5	.5	.5	.5	.5	.5	.5
Vitamin premix[d]	.5	.5	.5	.5	.5	.5	.5	.5
Trace mineral premix[e]	.1	.1	.1	.1	.1	.1	.1	.1
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[a]Moldy corn used in trials 1 and 2 was grown in 1976; that used in trial 3 was grown in 1977.

[b]Commercial product containing 18.5% Ca and 20.5% P.

[c]Contained .01% Co and .015% I.

[d]Amount per kilogram of premix: riboflavin, 880 mg; d-calcium pantothenate, 1.76 g; niacin, 3.95 g; choline chloride, 22 g; vitamin B_{12}, 3.95 mg; vitamin A, 660,800 IU; vitamin D, 132,000 IU; menadione sodium bisulfite, 440 milligrams.

[e]Amount per kilogram of premix; Mn, 60 g; Fe, 70 g; Cu, 10 g; Zn, 100 grams.

lated to provide 0, 3, 6 and 9 ppm of zearalenone in the complete diet. The diets were individually fed *ad libitum* in hoppers for 3 weeks and then at 2.3 kg/head once a day for another 3 weeks. Gain, feed consumption and visual appearance were monitored throughout the experiment.

The moldy corn was produced by artificial infection of the corn cob (Young, 1943) with Fusarium as the corn grew in the field during August of 1976. After harvest, the corn cobs were stored outside during the winter in a circular crib without cover. The corn was shelled the following spring and stored in a bin until used.

Assay of a sample of corn by analytical methods derived from the methods of Scott *et al.* (1970) and Stoloff *et al.* (1971) revealed a level of 39 ppm zearalenone.

Trial 2. Forty-eight pigs approximately 4 weeks of age were confined to 24 cages, with one barrow and one gilt per cage. The animals were fed *ad libitum* diets containing four levels of zearalenone (0, 3, 6 and 9 ppm) and two levels of cane molasses (0 and 5%) in a factorial arrangement for a period of 2 weeks. The diet formulas were the same as those used in trial 1. We added molasses to one-half of the diets to determine whether the factor(s) that may cause depressed feed intake could be masked.

All gilts were slaughtered at the end of the trial, and their uteri were removed, dissected free of excess tissue and weighed. In addition, segments from the midregion of the vagina and one uterine horn were fixed in Bouins solution and embedded in parafin wax. The tissues were cut into 5-μm sections and stained with hematoxylin and eosin. The slides were observed and photographed with a Zeiss photomicroscope.

Trial 3. Twenty-four gilts approximately 5 weeks of age were individually fed *ad libitum* diets containing four levels of zearalenone (0, 3, 6 and 9 ppm; table 1). The levels of zearalenone were achieved by replacing corn in the basal diet with zearalenone-contaminated corn grown in 1977. The contaminated corn, produced as in trial 1, contained approximately 107 ppm zearalenone. All the gilts were slaughtered at the end of the trial, and the reproductive tracts were evaluated as in trial 2.

Results

Trial 1. Rate of gain, feed consumption and gain to feed ratios during the full feeding period decreased with increasing levels of moldy corn in the diet (table 2). The effects, especially at the two highest levels of moldy corn, were observed within the first 3 days of the experiment, as indicated by weight loss, decreased feed consumption and a negative gain to feed ratio. Feed consumption during the first 3 days by pigs given the 6 and 9 ppm treatments was reduced to 22 and 42%, respectively, of that observed for pigs given the other two treatments. The reason for the greater consumption by the pigs offered the 9 ppm treatment than by those fed the 6 ppm treatment is unknown. It appeared that some form of adaptation occurred during the subsequent 18 days, because consumption of the diets containing the higher levels of moldy corn increased to a greater extent than did that of the diets containing the lower levels.

Regression analysis indicated that about 55% of the variation in average daily gain during the first 21-day period was accounted for by the variation in level of moldy corn in the diet.

During the 3-week limited feeding period, the level of moldy corn in the diet did not appear to have a consistent effect on rate of gain, feed consumption or gain to feed ratio (table 2). Although we had intended to maintain feed intake at 2.3 kg/head/day, some pigs did not consume all of the feed; thus, some adjustment of intake was necessary. Part of the apparent improved relative performance of pigs fed the two higher levels of moldy corn may be attributed to compensatory growth. Also, limit feeding would have tended to penalize to a greater degree the pigs fed the more palatable diets.

Increased redness and swelling of the vulva were observed in some gilts within 3 days after the start of the experiment. The differences tended to increase as the experiment progressed, but we could not readily distinguish treatment given by the symptoms of estrogenism observed. The absence of obvious differences between the groups may have been due partly to: (1) variation among animals in diet consumption and (2) reduced consumption of the diets containing the higher levels of moldy corn, resulting in similar consumption of total toxin (table 2).

Dietary treatment groups could not be differentiated by symptoms of estrogenism during the last 21-day limited feeding period. Some swelling of the vulva was observed in the gilts fed the 0-level of moldy corn. It is possible that some of the gilts were approaching puberty by the end of the trial. Although visual symptoms of apparent estrus were noted, checks with boars and by application of back pressure indicated that true estrus did not occur.

Assay of the moldy corn at a different laboratory[6] indicated levels of 19.5 ppm zearalenone and 37.5 ppm vomitoxin and nondetectable levels of mono- and diacetoxyscirpenol and T-2 toxin. No evidence of emesis was observed in these gilts.

Trials 2 and 3. The addition of 5% molasses to half of the diets in trial 2 did not have a measurable effect on the performance criteria evaluated; thus, only the effects of the levels of moldy corn are reported. In both trials, there was a significant linear depression in average daily gain as the level of moldy corn in the diet increased (table 3), although daily gains by the pigs fed the two intermediate levels of toxins appeared similar. Attempts were made to minimize and account for all feed wastage that occurred, but this was difficult, and, therefore, feed consumption data are not accurate. However, consumption appeared to be decreased by moldy corn, especially when the highest levels of toxins were fed. The superior performance of pigs in trial 3 as compared with trial 2 may have been due partially to (1) the higher level of protein in the diet, (2) the slightly lower levels of zearalenone fed to older pigs and (3) a different sample of corn.

Visual observation indicated some swelling and redness of the vulvae within 6 days after the start of the trials. All gilts fed 6 and 9 ppm zearalenone exhibited swollen and red vulvae 14 days after the initiation of the trials. Some of the gilts fed the 3 ppm zearalenone diets also exhibited redness and swelling of the vulvae. Two gilts fed the highest level of moldy corn in trial 2 exhibited vaginal prolapse by the end of the trial.

Uterine weights and uterine weights adjusted to the same body weight by covariance analysis increased linearly as the level of moldy corn in the diet increased. There appeared to be a

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TABLE 2. PERFORMANCE OF GROWING GILTS FED MOLDY CORN, TRIAL 1

Item	21-day full-fed period					21-day limit-fed period				
Level of zearalenone, ppm[a]	0	3	6	9	SD	0	3	6	9	SD
Age at start days	130	125	123	119	15					
Avg weight, kg										
Initial	63.8	63.6	63.8	62.6	10.0	84.0	79.8	69.2	66.8	11.9
Final	84.0	79.8	69.2	66.8	11.9	95.8	88.9	79.0	77.7	11.7
Avg daily gain (21 days), kg	.94	.77	.26	.20	.24	.56	.43	.47	.52	.10
Avg daily feed consumption (21 days), kg	3.02	2.72	1.28	1.72	.91	2.19	2.15	2.03	2.03	.12
Gain:feed	.32	.29	.14	.18	.29	.26	.21	.22	.26	.05
Calculated daily consumption										
Zearalenone, mg a	.0	8.13	7.65	15.53	4.75					
b	.0	4.06	3.84	7.76	2.37					
Vomitoxin c	.0	7.84	7.39	14.94	4.57					
Avg daily gain, kg										
Days 0 to 3	1.14	1.08	−1.06	−1.00	.66					
Days 4 to 21	.93	.72	.48	.40	.25					
Avg daily feed consumption, kg										
Days 0 to 3	2.83	2.84	.63	1.20	.33					
Days 4 to 21	3.05	2.71	1.39	1.81	.71					
Gain:feed										
Days 0 to 3	.41	.37	−2.36	−1.24	1.22					
Days 4 to 21	.31	.27	.35	.22	.28					

[a]Based on a level of 39 ppm zearalenone in the moldy corn.

[b]Based on a level of 19.5 ppm zearalenone in the moldy corn.

[c]Based on a level of 37.5 ppm vomitoxin in the moldy corn.

TABLE 3. PERFORMANCE OF YOUNG PIGS FED MOLDY CORN, TRIALS 2 AND 3

Item	Trial 2					Trial 3				
Calculated level of zearalenone, ppm[a]	0	3	6	9	SD	0	3	6	9	SD
Calculated level of vomitoxin, ppm[b]	0	2.9	5.8	8.7		0	.5	1.0	1.4	
Assayed level of zearalenone, ppm[c]						0	2.5	4.4	6.7	
Assayed level of vomitoxin, ppm[c]						0	<1	1.7	3.6	
Initial age, days	29	30	29	30		37	34	35	31	
Avg weight, kg										
Initial	6.3	6.3	6.3	6.3	1.1	7.0	6.6	6.7	6.2	1.9
Final	9.1	8.2	8.5	7.6	1.5	13.7	11.7	11.5	9.7	2.2
Avg daily gain, kg	.13	.09	.10	.06	.02	.32	.24	.23	.17	.06
Avg total feed consumption, kg	14.2	12.8	10.7	9.8	2.7	12.0	10.3	12.4	7.5	2.2
Calculated consumption										
Zearalenone, mg[d]	0	38.5	64.4	88.4		0	25.8	54.6	50.3	
Vomitoxin, mg[d]	0	37.2	62.0	85.3		0	<10.3	21.1	27.0	
Gain:feed	.20	.15	.22	.13	.05	.57	.50	.40	.44	.10
Uterine weight, g	2.5	4.3	9.2	11.3	1.8	4.3	6.8	10.6	15.5	4.5
Uterine weight:body weight[e]	.26	.49	1.06	1.36	.23	.32	.60	.97	1.46	.32

[a]Based on levels of 39 ppm, from one assay of the moldy corn in trial 2, and 107 ppm, the average of four assays in trial 3.

[b]Level of vomitoxin in moldy corn: trial 2, 37.5 ppm; trial 3, 17 ppm.

[c]Assays performed by Northern Regional Research Center.

[d]Calculated consumption based on calculated zearalenone and vomitoxin contents of diets in trial 2 and assayed levels in trial 3.

[e]Uterine weight in grams × 1,000 ÷ kilogram body weight.

closer relationship between uterine weight and dietary level of zearalenone than between other measures such as rate of gain or feed consumption and level of zearalenone.

In gilts given the two highest dosages (6 ppm and 9 ppm zearalenone), the myometrium and endometrium of the uterus essentially doubled in thickness. The increase was due to both hypertrophy and hyperplasia.

The vaginal epithelium ranged from a single layer of simple columnar or pseudostratified epithelium in the control gilts to a squamous epithelium six to 12 cells deep in the gilts fed high levels of moldy corn. Intermediate responses were observed in gilts in the lower dosage groups, with the epithelium sometimes increasing to three or four layers of cuboidal cells.

Discussion

A more consistent response in performance (gain, feed consumption, gain to feed ratio) due to level of dietary moldy corn was observed among the older pigs (trial 1 *versus* trial 2) fed similar diets. Forsyth *et al.* (1977) observed a high feed refusal rate when naturally infected corn containing 3.6 or 12 ppm dexoynivalenol was fed to 20-kg pigs. A level of 2.9 ppm toxin did not appear to influence feed intake in trial 1 and caused only a small reduction in trial 2; however, a level of 5.77 ppm reduced feed intake to 22% of that of controls (trial 1) during the first 3 days, and to 42% of the controls' intake during the 21-day trial. In trial 2, the reduction was to 88% of the controls' intake during the 14-day trial. Thus, the degree of feed intake reduction due to vomitoxin appears to be influenced by the age of the pig as well as the duration of the experimental period.

It would be difficult to attribute the decreased performance of pigs in trial 3 to level of dietary vomitoxin consumption, since the levels were estimated to be considerably lower than those in the previous two trials. The linear regression between daily gain and level of zearalenone needs to be interpreted with caution, since all other compounds unique to the moldy corn, identified or unidentified, would also be correlated. Christensen *et al.* (1977a) suggest that pure zearalenone, when added to the diet in a sufficient amount to cause severe estrogenism, does not cause stunting. However, Kotsonis *et al.* (1975) suggested that zearalenone together with one or more trichothecenes may cause feed refusal. Therefore, the specific factor or combination of factors that caused performance to be reduced with increasing levels of moldy corn in the diet are not known.

The similarity and linearity in response to calculated levels of dietary zearalenone and uterine weight:body weight in trials 2 and 3 are encouraging. Christensen *et al.* (1977b) suggested that "in the absence of contamination by diethylstilbesterol, zearalenone produced by Fusarium, and almost exclusively be *F. roseum*, is the only known cause of the estrogenic syndrome in swine."

The changes in the histology of the reproductive tract observed were similar to those reported by Kurtz *et al.* (1969). There was some variation in the degree of response among pigs fed the same diet, probably because of variation in intake of mycotoxin.

The variation in the analytical values of the toxin content of the same corn is of concern. It may have been due to poor sampling technique, to variations in technique between laboratories and(or) to analytical variation within laboratories. The diets were formulated on the basis of analytical values of samples of the whole corn. It is assumed that the analytical values obtained for the prepared feed are most representative of what was actually fed.

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